MOVEMENT, SPILLOVER AND GENE FLOW WITHIN A NETWORK OF NORTHERN MARINE RESERVES

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Abstract

The lack of empirical knowledge on how marine reserves should be effectively designed has impeded their extensive implementation as fisheries management tools. While current knowledge is largely based on computer models, few studies have verified them in the field. This study addresses several aspects of European lobster (*Homarus gammarus*) biology, directly related to future design of lobster reserves. First, spillover from reserves was measured by capture-mark-recapture studies. Second, probability of lobsters' presence within a reserve over time, along with their seasonal depth use, was quantified by acoustic telemetry. Third, connectivity of lobsters in Skagerrak was extrapolated from gene flow by microsatellite markers. Out of 2067 lobsters tagged within three reserves (0.5 km^2 - 1 km^2), only 3.6% was caught beyond reserve boundaries, with a mean spillover distance of 3.7 km. In comparison, 36.4% of tagged lobsters were recaptured within reserves. Moreover, individual lobsters were 50% likely to stay within a 0.5 km^2 reserve for a whole year. Males, and especially ovigerous females, used deeper habitats throughout the winter (≤ 58 m), compared to mean depth use (≈ 24 m). Furthermore, analyses of microsatellite markers revealed a subtle, although significant genetic structure (F_{ST} = 0.000, 95% CI: from 0.001 to -0.001, p = 0.039). Despite their small area, the reserves appeared to be sufficiently large to both contain a significant number of lobsters, and supply moderate levels of spillover to surrounding (fished) areas. Extensive gene flow within the study area indicates high connectivity. An extrapolation into effective migrants, done based on overall F_{ST} 95% confidence limits and effective population sizes ranging from 50 to 1000 individuals, showed that: at least 35 to 183 individuals were exchanged among sampled sub-populations every generation, and at most there was full exchange of migrants. Thus genetic data suggests that reserves could (hypothetically) exchange a demographically relevant number of migrants, given high effective population sizes within large reserves. These results could be of great importance for future design of reserve-networks containing species with a similar life history as the European lobster.

Acknowledgements

Management of marine resources is something we can and have to do better. I would like to thank several people for allowing me to take part in the scientific process of improving the management of the European lobster. First, I would like to thank Nils-Christian Stenseth for being my main supervisor at the University of Oslo. Second, I would like to thank: Esben Moland Olsen, Halvor Knutsen, and Even Moland at the Institute of Marine Research for being my CO-supervisors. They have invested many hours in my thesis, and provided me with plenty of well-sampled data. Also, they put great faith in me, by gradually increasing the grasp of my thesis. Without them I would not have been able to do this. Thank you also for the many fruitful discussions; the long drives to Flødevigen always paid off and gave me a lot to think about when driving back.

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Introduction

Over-exploitation characterises many commercially important marine species (Worm *et al.* 2009). A proposed benefit from marine reserves is to safeguard against unsustainable harvesting, while replenishing fished populations (Sale *et al.* 2005). Still, a lack of empirical data on how marine reserves should be effectively designed impedes their full-fledged implementation in fisheries management. Models suggest that up to 40% of a population's extent should be within reserves for optimal productivity (Pelc *et al.* 2010). Moreover, to ensure self-sufficiency within a single reserve, it has to encompass at least twice the alongshore distance of a mean larvae disperser (Lockwood *et al.* 2002). Alternatively, recruitment from fished areas has to be above ≈ 35% of natural levels (Botsford *et al.* 2001). Thus for overfished species with extensive larval dispersal, sufficient recruitment levels within reserves can only be sustained by an interconnected network of reserves (Gaines *et al.* 2010).

To a fisher, the primary positive effect of a marine reserve is dispersal of adults and juveniles into adjacent, fished areas (termed "spillover"). For example, spillover from a spiny lobster (*Palinurus elephas*) reserve sustained high catch rates 1.5 km into fishing grounds (Goñi *et al.* 2006). Despite loosing 32% of the local fishing grounds, spiny lobster catches increased with over 10% (Goñi *et al.* 2010). In contrast, a conservationist may argue for containing large, highly fecund individuals within reserves. This is to maximise production and subsequent export of pelagic larvae from reserves (termed "recruitment benefits") (Gell & Roberts 2003). To ensure both conservation and fisheries benefits of reserves, there should be a balance between containment and dispersal; a balance carefully regulated by reserve design (Halpern & Warner 2003). Even though larval dispersal kernels of most species of commercial species are largely unknown (Sale *et al.* 2005), measuring gene flow by neutral genetic markers appears to be a good approximation for both distance and magnitude of larval dispersal (Waples 1998; Palumbi 2003). Subsequently, estimates of larval dispersal can be used to optimise reserve placing, ensuring connectivity among reserves

(Palumbi 2004). For example, 10% of newly recruited anemone fish (*Amphiprion pelcula*) have been shown to originate from a reserve 35 km away (Planes *et al.* 2009).

Over-exploitation of European lobster (*Homarus gammarus*) throughout southern Norway has resulted in dismally low catches, compared to the mid 1900s (Agnalt *et al.* 2007). The severe reduction in population size has resulted in the listing of the European lobster as 'near threatened' in the Norwegian Red List (Oug *et al.* 2010). To ameliorate the Norwegian lobster stock, new legislative measures have recently been implemented. Amendments include: a reduction in number of traps allowed, an increased minimum size limit (25 cm total length), and immediate release of ovigerous females. This is on top of the limited fishing season from 1 October to 30 November. Further, in 2006, the Ministry of Fisheries and Coastal Affairs established four experimental lobster reserves throughout coastal Skagerrak. Their general aim was to determine how lobster populations would develop in the absence of fishing (Pettersen *et al.* 2009). Abundances of lobsters within these reserves have more than doubled, and the demography has shifted towards significantly larger individuals (Institute of Marine Research, unpublished data). Similar positive effects have been found in the Lundy lobster reserve in the UK (Hoskin *et al.* 2011).

The European lobster's rapid response to conservation makes marine reserve theory an eligible approach to their management. However, to ensure both fisheries and conservation benefits, key questions regarding effective lobster reserve design have to be answered. Here I quantify: lobsters' seasonal depth use and the probability of a reserve to contain individual lobsters over time (movement); spillover from reserves; and gene flow among sub-populations both partially within and outside reserves. This was to work out: the depth range to be included within reserves, and if reserves are large enough to contain a significant number of lobsters over time; the spatial extent and magnitude of spillover from reserves; and if there is sufficient gene flow in Skagerrak for reserves to exchange sustainable levels of recruits. A wide framework of methods was used when addressing these questions, easily applicable to novel reserve design challenges: movement was quantified by acoustic telemetry; spillover was measured by capture-mark-recapture (CMR) studies;

and connectivity was extrapolated from gene flow by microsatellite markers. To the best of my knowledge, no European study have evaluated the design of a network of marine reserves separated by 20 km - 300 km, factual distances among experimental lobster reserves. My results could therefore be of great importance for future design of reserve-networks containing species with similar life history as the European lobster.

Materials and methods

Study species

The European lobster *Homarus gammarus* (Linnaeus 1758) is a large decapod (Fig. 1A) distributed from northern Norway to Morocco. Female lobster reproduction cycle generally lasts two years. They moult and mate the first summer, and extrude their eggs the following summer. Eggs hatch the next summer, after which the females immediately moult and mate (Agnalt *et al.* 2007). Females carry eggs from 9 to 11 months, and eggs need an excess of 2772 degree-days to complete embryonic development (Schmalenbach & Franke 2010). However, embryonic development stops at temperatures below 3.4°C (Campbell & Stasko 1986). In their northern extent, hatching usually occurs within the period from April to July (Schmalenbach & Franke 2010), peaking around summer solstice (Ennis 1973). The four subsequent pelagic larval stages are predominantly found in the neuston, where they display a strong diel vertical migration (Nichols & Lovewell 1987). When suitable habitat is found during stage four they settle to the bottom, preferably among rocks (Cobb & Wahle 1994). Settlement follows 13 to 35 days in the pelagic, depending on temperature (Schmalenbach & Franke 2010). However, there have been few observations of early benthic phase lobsters in the field, and their biology is largely unknown (Linnane *et al.* 2001).

Study site

The data analysed in this study were sampled at three spatial levels along the Skagerrak coast. At the largest scale, I studied the genetic structure of the lobster in Skagerrak (Fig. 1B). I sampled individuals for microsatellite markers at two sites on the Swedish part of the Skagerrak coast (Kåvra and Gullmaren) and six sites along the Norwegian Skagerrak coast (Tisler, Singlefjorden, Inner Oslofjorden, Bolærne, Flødevigen, and Mandal). Of these locations, Kåvra, Flødevigen, and Mandal were relatively exposed to the Norwegian coastal water (NCW) current. The NCW moves in a counter clockwise gyre along the Skagerrak coast (Kåvra situated the furthest 'upstream') at mean speeds of 10 - 40 cm^{-s} during summer, weakly dissipating in deeper layers (Danielssen *et al.*

1997). Tisler and Bolærne were situated further away from the NCW current, though exposed to a small gyre in the outer Oslofjord. The remainder locations: Gullmaren, Singlefjorden and Inner Oslofjorden were situated inside their respective fjords. Additionally, there was a significant input of Jutland coastal water from southwest into Skagerrak. Skagerrak surface water was probably influenced by wind conditions, highly variable in the 'dispersal window' of the lobster larvae. Also, a decreasing horizontal salinity gradient is present towards southeast of the general study area (Nielsen 2005a). Moreover, the coastal strip of Skagerrak is divided into an eastern and western part by a 200 m - 300 m deep gorge across the outer Oslofjord.

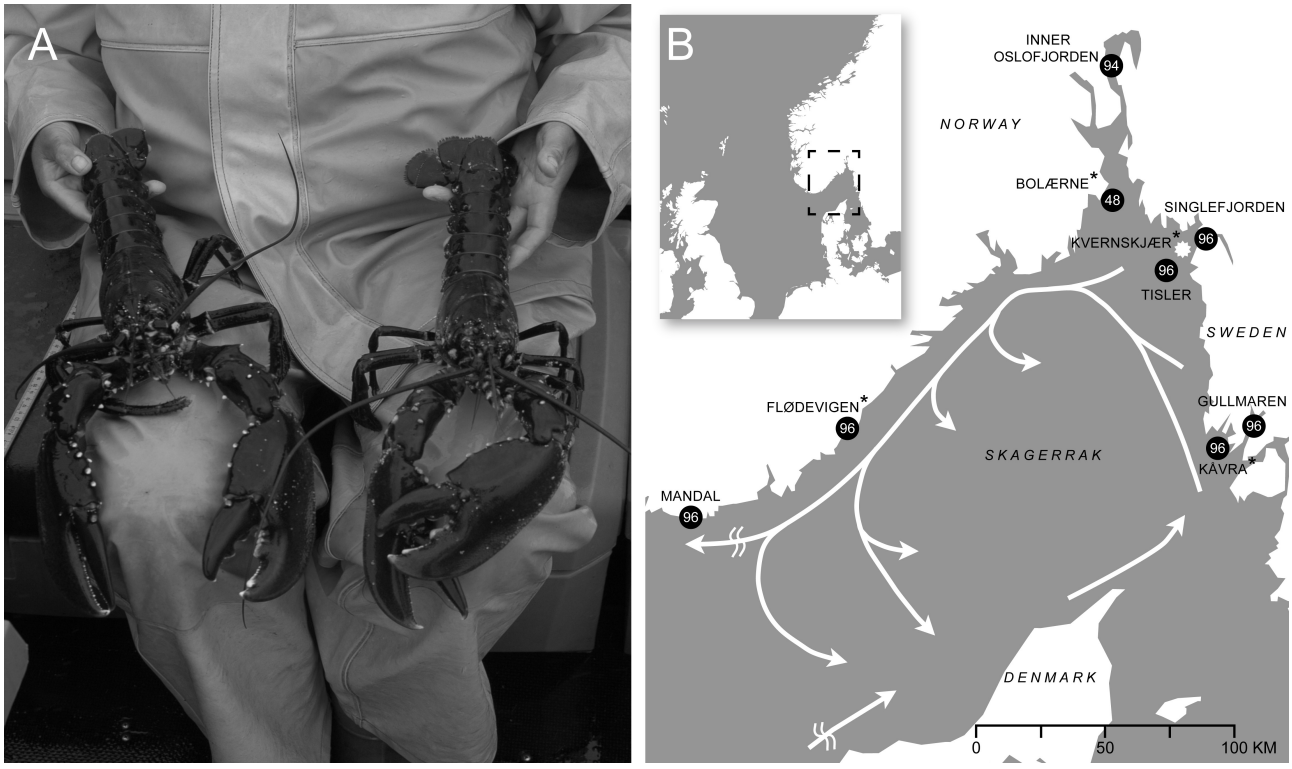

Figure 1 (A) Picture of what is now considered two decent sized European lobsters, caught within the Kvernskjær lobster reserve. (B) Illustration of Skagerrak Sea, depicting all sampling sites and sizes for microsatellite markers (black circles with numbers) and telemetry study site (white star). Sites with asterisk (*) indicate locations of lobster reserves. White arrows represent the prevailing currents of the Skagerrak: the Norwegian coastal water current (which continues along the Norwegian coast) and input from the Jutland coastal current. Currents are redrawn from Danielssen *et al.* (1997).

When measuring spillover, the focal areas were the lobster reserves of Kvernskjær, Bolærne and Flødevigen (Fig. 1B). These three lobster reserves varied in size, approximately 0.5 km^2, 0.7 km^2 and 1 km^2. The reserves harboured typical lobster habitat representative of coastal Skagerrak, which was one of the selection criteria used when the experimental reserves were established (Pettersen *et al.* 2009).

At the smallest scale, I studied movement of lobsters within the Kvernskjær lobster reserve. This reserve was situated around a small island/skerry in the Hvaler archipelago, flanked by a particularly steep slope and deep ravine (≤60 m) on the western side (Fig. 2A). SCUBA surveys performed in the area before reserve establishment revealed that macro-algae were sparse near the surface. This was probably due to constant discharge of fresh water from the nearby Glomma River. However, macro-algae were present from 5 m - 12 m, and the submerged plateau at the southern end of Kvernskjær contained a sparse kelp forest. The topography within the reserve could be delineated as typical of glacial scouring, containing rock faces and ledges with boulder fields at their base. In deeper basins and flat areas the bottom consisted of soft sediments and mud.

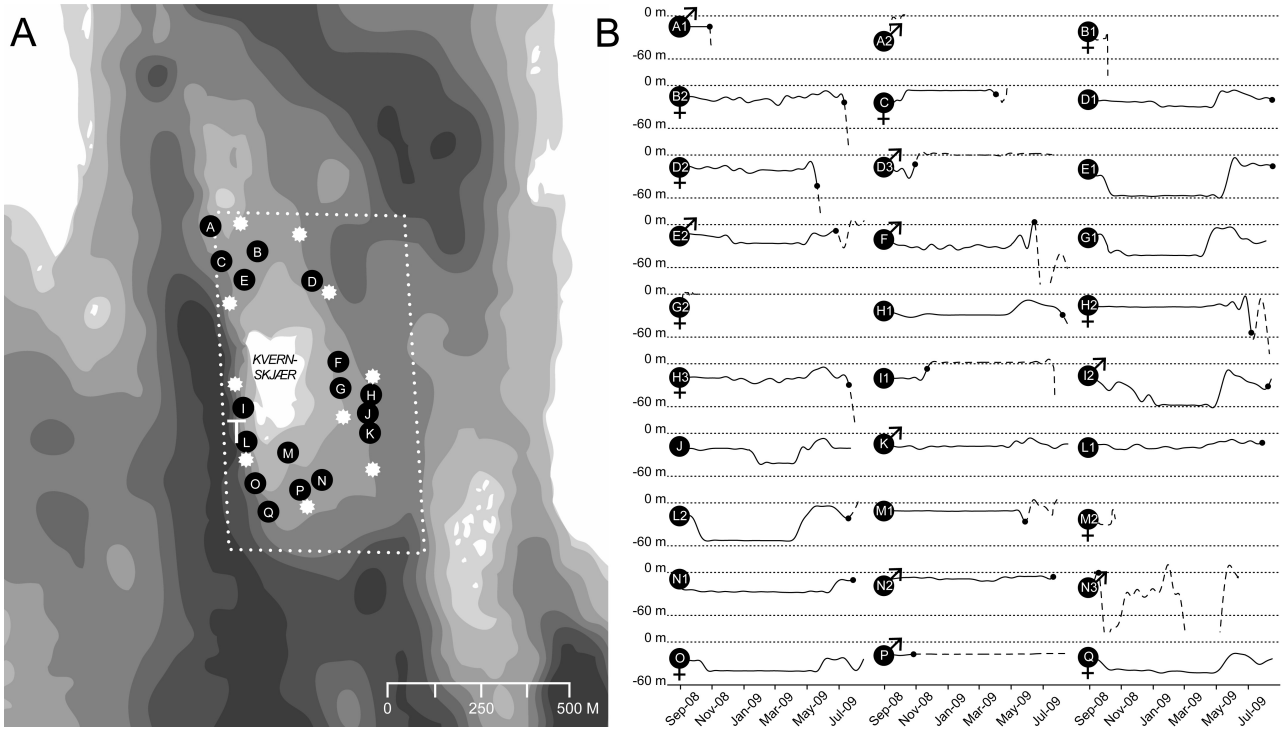

Figure 2 (A) Map of the Kvernskjær lobster reserve (white star symbol in figure 1B), delineating bathymetry of the telemetry study area, border of lobster reserve (white dotted line), grid of VR2W receivers (white stars) and release sites of lobsters (black circles). The ravine to the west of reserve was up to 60 m deep, and every change in grey represents a 10 m isobath (except the lightest, which includes the depth range 1 m - 0 m). White "T" indicates where the string of temperature loggers was deployed. (B) Smoothed depth data of individual lobsters fitted with acoustic transmitters throughout the telemetry study period (27 August 2008 to 24 August 2009). Broken lines indicate when individuals have been censored. Dotted lines indicate surface and maximum depth of study area, 0 - 60 m. Letters within black circles identify release site in panel A and arrows and crosses indicate male and female lobsters, whereas plane circles indicate ovigerous females.

Tagging and DNA extraction

The lobsters, for which movement (i.e. containment probability and seasonal depth use) was quantified, consisted of a sample of 10 ovigerous females, 10 non-ovigerous females and 10 males (Tab. 1). They were caught using mackerel baited parlour traps, deployed at depths between 10 m - 30 m, and soaked overnight on the 24, 25, and 26 August 2008. Based on visual appearances of their exoskeletons, recently moulted individuals were selected. Following the procedure described

by Aiken (1973), the individuals were moult staged by visual inspection of the outer segment of one of their pleopods in a dissection microscope. Subsequently, they were classified into one of four moult stages: C_4, D_0, D_1, or D_2. C_4 is traditionally termed the intermoult stage (anecdysis), a period where there is no physiological changes in moulting. C_4 glides into the D_0 stage (first stage of proecdysis), where the epidermis retracts from the cuticle. In D_1 new setae starts to form within invaginations of the epidermis. D_2 is defined as the stage where secretion of the new cuticle has started, and a new, pigmented layer is formed (Aiken 1973). Sex was determined by examination of the first pair of pleopods. Visual inspections confirmed that eggs of all ovigerous females were in development stage one, of four possible stages (Pandian 1970). Hence, the ovigerous females would not moult until the following year after the hatching of their eggs. Before release, the selected lobsters were brought on board the R/V G.M. Dannevig and equipped with V13P-1H high power coded acoustic transmitters with depth sensors (Vemco Ltd., Halifax, Canada, diameter 13 mm, length 45 mm, weight in seawater: 6 g, emitting on 69 kHz). Tags were set to transmit at a randomized interval between 110 and 250 s, and had an expected battery life of ≈ 500 days. A tag harness was constructed by inserting the tag into a soft plastic tube with superglue added to its internal surface. A plastic strip was then threaded through two holes punched in the piece of tubing. The harness was subsequently fitted to the middle segment of the crusher claw, with the depth sensor pointing posteriorly. This was to minimize the probability of damage to the sensor through wear during excavation and entering or exiting of shelters. Though, lobsters would eventually lose the acoustic transmitter during their subsequent moulting period. The actual tag-attachment procedure did not exceed five minutes. After the procedure, lobsters were released at their respective capture positions within the reserve (Fig. 2A).

Table 1 Summary for lobsters used in the telemetry study, including: code of individual (corresponding to release site in figure 2); carapace length (CL); total length (TL); group (male, female or ovigerous female); moult stage at start of study; number of times (N) the individual has been captured during standardized experimental fishing within Kvernskjær lobster reserve; and days since last captured within reserve.

Individual	*CL (mm)*	*TL (mm)*	*Group*	*Moult stage*	*N × captured in reserve*	*Days*
A1	86	241	*M*	C_4	1	68
A2	100	287	*M*	D_1	1	20
B1	86	252	*F*	C_4	1	9
B2	76	220	*F*	D_0	1	326
C	120	335	*F*	D_2	1	225
D1	89	253	*O*	C_4	2	363
D2	79	230	*F*	C_4	1	274
D3	84	239	*M*	C_4	1	70
E1	81	238	*O*	C_4	3	364
E2	122	342	*M*	C_4	2	310
F	98	280	*M*	D_1	2	299
G1	86	249	*O*	C_4	2	364
G2	89	255	*F*	D_0	1	9
H1	86	241	*O*	D_0	3	353
H2	92	263	*F*	C_4	3	323
H3	85	247	*F*	D_0	1	335
I1	84	247	*O*	C_4	3	93
I2	92	257	*M*	D_0	3	355
J	88	250	*O*	C_4	2	349
K	112	315	*M*	D_2	1	364
L1	89	250	*O*	D_1	1	344
L2	98	273	*O*	D_0	1	334
M1	84	247	*O*	D_2	3	281
M2	105	301	*F*	D_2	2	1
N1	92	265	*O*	C_4	2	343
N2	89	256	*M*	C_4	2	335
N3	94	262	*M*	D_1	2	30
O	96	278	*F*	D_0	8	364
P	86	245	*M*	D_0	2	67
Q	85	242	*F*	D_0	2	364

As part of the monitoring of the experimental lobster reserves, the Norwegian Institute of Marine Research has carried out standardized experimental fishing surveys inside the Bolærne, Flødevigen and Kvernskjær reserves. This has been an annual event since 2004, and data collected until fall 2010 was used in this study. As part of this survey, using the same trapping procedure as

described above, 2067 lobsters have received T-bar anchor tags (TBA1, 30 x 2 mm, Hallprint Pty. LTD, Holden Hill, South Australia) with printed information about the experimental fishing survey. The T-bar anchor tags were inserted in the ventral musculature between the cephalothorax and first abdominal segment, to the right side of the midline, using a standard tag applicator. This particular technique ensures retention of the tag through subsequent moulting (Agnalt *et al.* 2007). After having received their T-bar tags, lobsters were released at their respective trapping positions within the reserves.

DNA of individual lobsters was isolated from muscle tissue of the pleopod. Following the protocol described in André & Knutsen (2010), 12 microsatellite loci were amplified using PCR. These included *HGC131, HGC120, HGC111, HGD111, HGD106, HGC118, HGC103, HGB4, HGA8, HGC129, HGB6* and *HGC6*. The microsatellite DNA fragments were separated on an automatic sequencer (Beckmann Coulter SEC 8000), and classified according to nucleotide length. Two trained persons analysed the scorings independently. In case of disagreements, the individuals were re-analysed.

Study design

During the period 27 August 2008 to 24 August 2009, 10 VR2W receivers (Vemco Ltd, Halifax, Canada) were deployed to monitor the 30 lobsters fitted with acoustic transmitters and depth sensors (Fig. 2A). If a lobster had at least one VR2W receiver within its transmitter's broadcasting radius, its depth-at-time was stored along with an ID code within the receiver. VR2W receivers were moored to concrete elements with a rope, and kept erect at 9 m below surface by a buoy attached 1 m above it. To reveal sites in the study area where transmitted signals could not be received, a range test was conducted. This test was run as systematic transects throughout the study area, lowering a range-testing transmitter (V16-4H, Vemco Ltd.) down to the bottom at 70 positions. The range-testing transmitter broadcasted an ID code every 5 s with the same output power as the transmitters used in the telemetry study. This transmitter was attached to a rope

directly above a 10 kg weight. When the weight hit the bottom, time and GPS coordinates were noted, and the range-testing tag was left on the bottom for one minute. Whether receivers had registered any entries around the noted bottom time of the trial transmitter, determined if the given position was within a receivers listening area. Preliminary analysis of the range testing data indicated that shallow and marginal areas of the study area had the least "coverage", that is: positions within few or none of the receivers listening areas. To determine if seasonal depth use of the lobsters could be related with temperature, a string of temperature loggers (HoBo UA-001-08, Onset Computer Corp., Pocasset, Ma., USA) were deployed in the telemetry study area (white "T" in figure 2A). They recorded temperature at depths 7.5 m, 12.5 m, 17.5 m, 22.5 m, 27.5 m and 32.5 m, during period 21 November 2008 to 24 August 2009.

To get a measure of spillover of lobsters from reserves, we depended on local fishers to report any lobsters caught outside the reserves carrying T-bar tags. As it was assumed that fishers do not lay traps within the lobster reserves, a tagged individual caught by a fisher was a spillover lobster *per se*. If trapping location was provided by the fisher, the spillover distance of the lobster was measured as a straight line over water, from the reserve boundary to the reported trapping location. If landmasses intervened, the shortest way around was measured.

Moreover, to address connectivity in coastal Skagerrak, 718 lobsters were screened for genetic variability using the microsatellite markers (Fig. 1B). Lobsters sampled in Flødevigen, Bolærne and Kåvra where caught within their respective lobster reserves, whereas samples from the other five sites where bought from fishers in the area. Temporal replicates were taken one to three years apart. Due to their geographical proximity, we defined pairs: Bolærne, inner Oslofjorden; Tisler, Singlefjorden; and Kåvra, Gullmaren as within each other's domain.

Data analyses

Telemetry monitoring data was analysed in program R (R Development Core Team 2012). I categorized each individual's daily status as: present and moving within the study area, lost from the study, or censored. Lobsters in the first mentioned category had a depth signature that was considered 'normal'. For lobsters in the second category, the reason for a stop in the received signals was not known. Nevertheless, the individual was no longer in the study. Individuals categorised as censored typically emitted readings 2 m above, and 110 m below sea level within short periods of time. To predict the probability of containing telemetry tagged lobsters within the Kvernskjær reserve over time; I fitted Kaplan-Meyer survival curves to the data described above (Kaplan & Meier 1958; Cox 1972). For this purpose, the R library "survival" was used. Here a 'loss' curve was produced for: all individuals; one for each of the three groups ovigerous females, non-ovigerous females and males; and individuals categorized according to moult stage at study start. If moulting were the primary causation for transmitter loss, I would expect individuals classified into later moult stages at study start to be lost from the telemetry study earlier. To test if loss curves from the different categories were statistically equivalent; the log-rank test was used. Also, to determine if days in telemetry study was correlated with number of times individuals had been captured during experimental fishing, Kendall's rank correlation τ was used. Furthermore, to picture the temporal trends in each individual's depth use, cubic regression splines (Wood 2006b) were fitted to mean daily depth recordings from telemetry tagged lobsters. To ensure a comparable smoothness of all individual's depth signatures, the smoothing factor k was set to 30. Further, to determine if there was a difference in seasonal depth use among ovigerous females, non-ovigerous females and males, a generalized additive mixed model (GAMM) was fitted to the depth data (Wood 2004, 2006a, b). Here *Depth* (the mean daily depth of each individual) was used as response variable, with covariate *Days* (days into study period, continuous variable) by *Group* (factor with 3 levels, ovigerous females, females and males), yielding one smoother for each level in *Group* (i.e. a variable coefficient model). Thin plate regression splines were used as the smoothing algorithm,

while k was determined by the data by cross-validation (Wood 2003). Additionally, *Individual* (factor with 30 levels) was included as a random intercept in the model. Because a time series registered from a lobster were repeated observations of the same individual, temporal auto-correlation of the residuals was to be expected. Correlated error structures were modelled with an auto-regressive model of order 1 (AR-1). R package "mgcv" was used to fit the model (Eq. 1). Moreover, to model temperature in the telemetry study area over time, a two-dimensional, thin regression spline was fitted (by cross validation). Here *Temperature* (daily mean temperature at six depths) was modelled as response to the interaction between *Days* (days into study period) and *Depth* (depths 7.5m, 12.5m, 17.5m, 22.5m, 27.5m and 32.5m) (Eq. 2). It should be noted that temperature was only available for a limited part of the telemetry study period (21 November 2008 - 24 August 2009).

(1) $$Depth_{ij} = f_1(Days_i) \times Group_{1,i} + f_2(Days_i) \times Group_{2,i} + f_3(Days_i) \times Group_{3,i} + b_j \times Individual_j + \varepsilon_{ij}$$

$$b_j = N(0, d^2)$$

$$\varepsilon_{ij} = N(0, \sigma^2)$$

$$cor(\varepsilon_s, \varepsilon_t) = \rho^{|s-t|}$$

(2) $$Temperature_{ij} = f(Days_i, Depth_j) + \varepsilon_{ij}$$

$$\varepsilon_{ij} = N(0, \sigma^2)$$

From CMR data I tested if spillover distance of male and female lobsters was statistically different. Here the two-sample Wilcoxon rank sum (Mann-Whitney) test was used. Also, to determine if spillover distance was correlated with days at liberty, Kendall's τ was used. Both tests were available in R's base distribution.

From microsatellite markers I quantified deviations from Hardy-Weinberg (HW) equilibrium, using the F_{IS} statistic per sample site. To statistically test if sampled sites had excess or deficiencies of heterozygotes, I used two-sided HW probability tests. To estimate the proportion of

genetic variation that could be allocated among geographic samples, I used Weir & Cockerham's (1984) F_{ST} estimator θ. Standard errors of F_{ST} estimated for each loci were produced by jackknifing over alleles. Also, by bootstrapping over loci, I produced a standard error of the overall, mean F_{ST}. Furthermore, I estimated pairwise F_{ST} both between temporally replicated samples and geographic samples. In addition, I performed allele and genotype frequency tests of differentiation, both within loci and between sub-population pairs. F_{ST} with standard errors were calculated using the FSTAT software (Goudet 1995); and HW disequilibrium tests, F_{IS}, pairwise F_{ST} and allele/genotype frequency tests were calculated using software GENEPOP (Rousset 2008). As HW disequilibrium and allele/genotype frequency tests were a sequence of independent tests, their resulting p-values were adjusted using the False Discovery Rate (FDR) correction (Benjamini & Hochberg 1995). In preliminary analyses, linkage disequilibrium and presence of null alleles was ruled out, using programs FSTAT and MICRO-CHECKER (Van Oosterhout *et al.* 2004). Also, I confirmed that none of the microsatellite loci were under natural selection (Beaumont & Nichols 1996), using program LOSITAN (Antao *et al.* 2008). To visualise the variation in pairwise F_{ST} among temporally replicated samples, a non-metric multidimensional scaling (NMDS) diagram was made. The two-dimensional NMDS diagram was subsequently rotated to principal components, and units on axis were converted to half-change units. R package "vegan" was used to make the NMDS diagram. In parallel, an Analysis of Molecular Variance (AMOVA) was done to partition the variation within (i.e. between temporal replicates), and among geographic samples. The ANOVA was calculated in the software ARLEQUIN (Excoffier & Lischer 2010). Furthermore, to determine where largest discontinuities occur in the genetic landscape of lobsters in Skagerrak, 12 matrices containing per locus pairwise F_{ST} were analysed in program BARRIER (Manni *et al.* 2004). Here one barrier was computed for each locus. The consensus diagram for the 12 loci was superimposed on a diagram from an analysis done on the overall pairwise F_{ST} matrix. In the initial triangulation of the sample site coordinates virtual points were placed so that the Delaunay triangulations between inner Oslofjorden, Singlefjorden; Singlefjorden, Gullmaren; and Tisler, Gullmaren were removed.

The rationale was that land masses intervened between these sampling sites. Moreover, to estimate the mean number of effective migrants exchanged by sub-populations per generation, I used Wright's (1943, Eq. 16) formula of F_{ST} under the island model (Eq. 3), solving for N_em. Point estimates of N_em were also done based on the 95% CI of the overall F_{ST}. I assumed a range of effective population sizes N_e, including 50, 100, 500 and 1000.

$$N_e m = N_e \left(1 - \sqrt{\frac{2N_e F_{ST}}{[(2N_e - 1)F_{ST} + 1]}} \right) \quad (3)$$

Results

Containment and depth use of lobsters within the Kvernskjær reserve

After 363 days, half of the individuals fitted with acoustic transmitters had been lost from the study. During the period September to May there were few losses, while losses intensified during period July to August (Fig. 3A). In particular, ovigerous females had no losses from September to June, but considerable losses from July to August (Fig. 3B). However, according to the log-rank test, there was no significant difference in loss-curves among the three groups (χ^2 = 0.6 on 2 df, p = 0.741). Also, there was no significant effect of moult stage (classified at the start of the study) on loss curves (χ^2 = 1.7 on 3 df, p = 0.643); however, the group staged to the D_2 stadium had the lowest proportion left until very late in the study (Fig. 3C). There was a significant, positive correlation between number of days in the telemetry study and number of times the same individual have been captured within Kvernskjær reserve throughout experimental fishing (τ = 0.28, z = 1.97, p = 0.04).

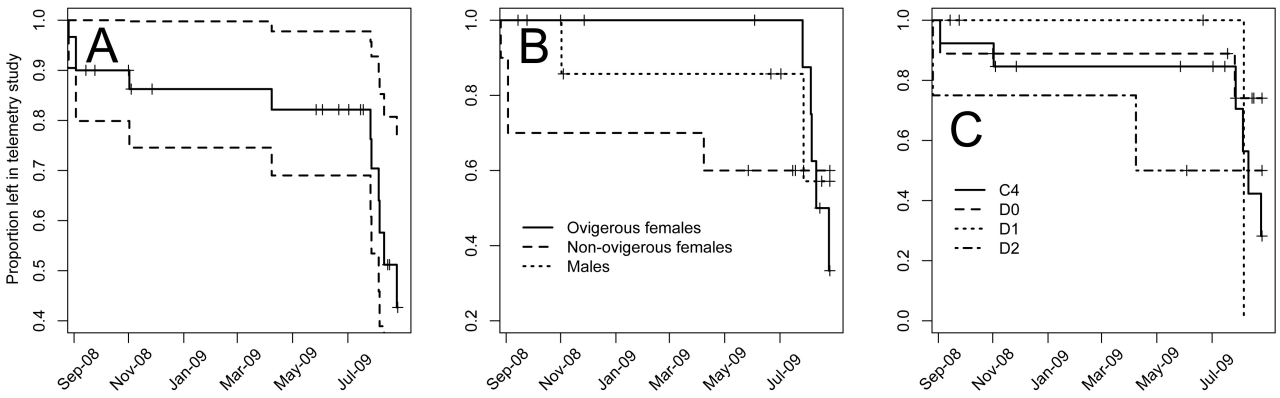

Figure 3 (A) 'Loss-curve' (Kaplan-Meyer curve) of all individuals in the telemetry study (with predicted 95% CI), where the proportion of individuals left in the study is plotted against the date of a given event. Crosses indicate right-censored individuals (i.e. where the depth sensor emitted nonsense depth readings and the individual was out of the study). In panel B there is one loss-curve for each of the three groups ovigerous females, non-ovigerous females, and males. In panel C there is a loss-curve plotted for each moult stage at start of study.

Observed surface temperatures (7.5 m) varied from almost 0°C in middle of February, to 20°C at start of July. Bottom temperatures (32.5 m) varied from a minimum of ≈ 4°C in end of March, to a maximum of 16.5°C in end of July. Predictions from the temperature model indicated that upper water masses had colder temperatures than lower water masses until April - May. In start of May, all depth strata had the same temperature (≈6°C). From late May, the upper strata had higher temperatures than lower strata (Fig. 4A).

At least nine individual lobsters distinctively shifted towards deeper habitats during winter (e.g. individuals: D1, E1, E2, G1, I2, J, L2, O, and Q in Fig. 2B). Furthermore, nearly all individuals briefly shifted to shallower habitats (≈0 m - 5 m) in spring and early summer. While some individuals were either within the study area for a short period, or had a nearly linear depth use throughout the year.

Predictions from the GAMM fitted to mean daily depth data suggested that ovigerous females moved to significantly shallower habitats than non-ovigerous females during period June - July (*per se*, due to their non overlapping confidence intervals during this period) (Fig. 4B). Mean predicted difference in ovigerous and non-ovigerous female lobster depth use was 11 m and 8 m for June and July respectively. Males seemed to follow the seasonal movement of ovigerous females to some extent. However, ovigerous females moved to shallower depths than males during summer (mean difference of 9 m in June), and slightly deeper habitat during winter (mean difference of up to 5 m, in November). Predicted depth use of non-ovigerous females was nearly linear throughout the study period. During fishing season (from October to November) there were no characteristic differences in predicted depth use among the three groups. However, this appeared to be a period of much movement, as all three groups were on the move towards deeper habitats. Moreover, the GAMM could explain 18% of the variation in data (adjusted R^2), and all three smooth terms were significant (edf = 7.1, F = 14.7, p = <0.001; edf = 2.1, F = 3.1, p = 0.045; edf = 4.2, F = 4.1, p = 0.003 for ovigerous, female and male lobsters). Residuals of the GAMM separated by one time unit (day) had an auto-correlation of 0.95.

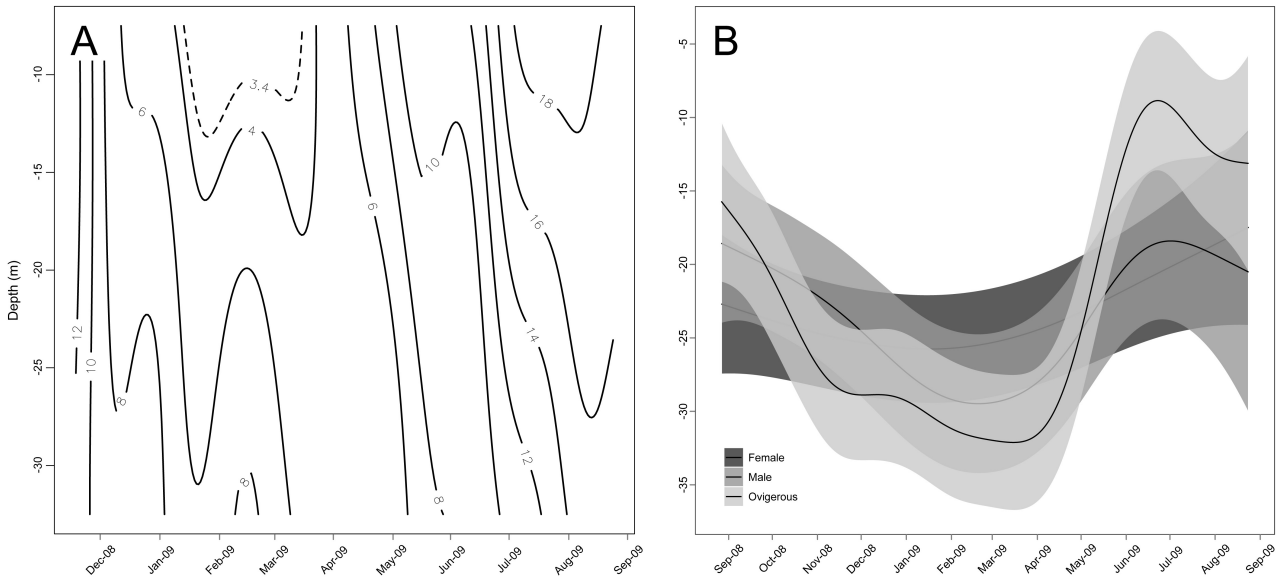

Figure 4 (A) Two-dimensional smoother of temperature throughout time period 21 November 2008 - 24 August 2009 predicted for the depth interval 7.5 m - 32.5 m. Isolines connects depths of equal predicted temperature. (B) Prediction from the GAMM fitted to daily mean depth of lobsters carrying depth sensors, with one smoother for each group: ovigerous, female, and male lobsters (with corresponding 95% CIs).

Spillover of lobsters from Skagerrak reserves

Of 2067 lobsters (51 mm - 149 mm CL) tagged and released within three of the lobster reserves during experimental fishing surveys, 75 (3.6%) have been caught and reported by fishers outside the reserves. In comparison, 752 (36.4%) individuals were recaptured at least once inside the reserves during experimental fishing (Tab. 2). Size range of spillover lobsters was 83 mm - 159 mm (CL), and the mean distance these individuals were displaced was 3.7 km (N = 43). However, the distribution of recapture distances from reserves was highly skewed (Fig. 5A). Furthermore, there was no difference in spillover distance between males and females ($W = 255.5$, $p = 0.75$) (Fig. 5B). Neither was number of days lobsters had been at liberty correlated with displacement distance ($\tau = 0.09$, $z = 0.92$, $p = 0.35$).

Table 2 Capture-mark-recapture data from three lobster reserves throughout six years of experimental fishing, including: number of individuals tagged within reserve (N) recaptured (R) at least one time within (W) reserves (R|W), or recaptured by fishers (F) outside the reserve (R|F); proportion of individuals (Pr) tagged within reserves recaptured within reserve at least one time (Pr|W), or recaptured by fishers outside reserve (Pr|F); mean horizontal displacement; total length (TL); and days since last recapture within the reserve of individuals recaptured by fishers.

						Mean|F		
	N	*R|W*	*R|F*	*Pr|W*	*Pr|F*	*Displacement (km)*	*TL (mm)*	*Days*
Kvernskjær	641	262	16	0.409	0.024	3.6	271	372
Flødevigen	579	250	24	0.432	0.041	2.2	292	249
Bolærne	847	240	35	0.283	0.041	4.9	282	370
Total	2067	752	75	0.364	0.036	3.7	283	331

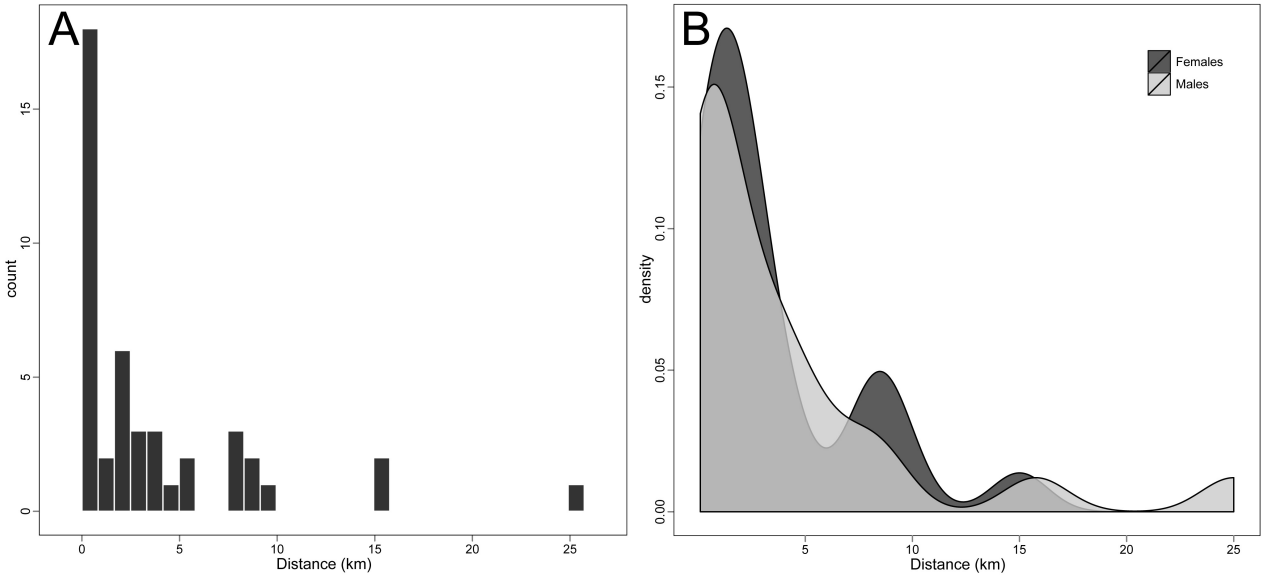

Figure 5 (A) Number of tagged lobsters throughout experimental fishing surveys that have been caught and reported by fishers outside Kvernskjær, Bolærne, and Flødevigen lobster reserve. Individual spillover distance is categorised into intervals of 1 km. (B) Kernel density plot of same data as in panel A, although categorised into males and females.

Gene flow of lobsters in Skagerrak

The proportion of genetic variation that could be partitioned among geographic samples (F_{ST}), estimated for each locus, ranged from -0.003 to 0.002 (Table 3). The mean F_{ST} across all loci was estimated to 0.000, with 95% confidence limits ranging from 0.001 to -0.001. Overall, allele frequencies varied significantly among geographic samples, also after FDR corrections (p=0.039). When extrapolated into effective number of migrants exchanged among sub-populations per generation (N_em), at least: 34, 58, 146, and, 183 individuals were exchanged. These estimates were based on the lower 95% confidence limit of the overall F_{ST} (0.001), and effective population sizes (N_e): 50, 100, 500, and 1000. On the other hand, since the upper 95% confidence limit of the overall F_{ST} was negative (-000.1), its respective N_em could best be described as free exchange of migrants. Based on the overall mean F_{ST} (0.000), N_em would be the same as N_e (i.e. all individuals in sub-populations were migrants/free exchange of migrants). Accordingly, sub-populations would exchange at least 34 to 183 individuals, and at most there would be full exchange of migrants among the sub-populations.

Table 3 Summary statistics of genetic variability within locus, including: locus name; number of alleles at locus; average heterozygosity at locus (H_T); proportion of genetic variation partitioned among geographic samples (F_{ST}, with corresponding 95% confidence limits); p-values from allele frequency tests; and FDR corrected p-values from allele frequency tests.

Locus	*Alleles*	H_T	*Upper 95% CI*	F_{ST}	*Lower 95% CI*	*P-value*	*FDR corr.*
HGC131	23	0.849	0.004	0.002	0.000	0.006	0.072
HGC120	18	0.860	0.002	0.000	-0.002	0.192	0.384
HGC111	14	0.755	0.004	0.000	-0.004	0.242	0.414
HGD111	14	0.602	0.006	0.002	-0.002	0.299	0.448
HGD106	11	0.685	0.000	-0.002	-0.004	0.503	0.565
HGC118	10	0.552	0.004	-0.000	-0.004	0.074	0.222
HGC103	9	0.691	0.004	-0.000	-0.004	0.518	0.565
HGB4	7	0.642	-0.001	-0.003	-0.005	0.971	0.971
HGA8	14	0.808	0.002	0.000	-0.002	0.043	0.216
HGC129	10	0.588	0.001	-0.001	-0.003	0.054	0.216
HGB6	11	0.791	0.001	-0.001	-0.003	0.395	0.526
HGC6	10	0.384	0.003	0.001	-0.001	0.115	0.276
Overall			0.001	0.000	-0.001	0.006	

According to their pairwise F_{ST}, temporal replicates from inner Oslofjorden, Kåvra, Gullmaren, and Flødevigen were placed close together along the first axis of the NMDS diagram. While Singlefjorden, Tisler, and Mandal temporal samples were placed close together along the second axis. Judged by the units on the axes, they represented an equal amount of variation in pairwise F_{ST} (Fig. 6A). However, the AMOVA did neither partition a significant amount of variation between temporally replicated samples, nor among sub-populations. Accordingly, most genetic variation (≈99%) was found within samples. Moreover, of the eight sub-populations sampled for microsatellite markers, four had high probabilities ($p < 0.05$) of a deviation from HW equilibrium expectations. After FDR corrections, only three significant p-values remained (Tab. 4). Though, within each sub-population, only two loci showed significant p-values at most.

Table 4 Summary statistics of genetic variability within geographic samples, including: average heterozygosity (H_S); allelic richness; and HW disequilibrium within each sampled site measured as F_{IS}, along with p-values from probability tests (H_1 = excess or deficiency of heterozygotes) and their FDR corrected p-values.

			HW disequilibrium		
Sample site	H_S	*Allelic richness*	F_{IS}	*P-values*	*FDR corr.*
Gullmaren	0.698	8.101	-0.009	0.288	0.384
Kåvra	0.669	8.218	0.024	<0.001	<0.001
Tisler	0.650	8.152	0.041	0.027	0.055
Singlefjorden	0.664	8.008	0.021	0.268	0.384
Inner Oslofjorden	0.664	8.528	0.034	<0.001	<0.001
Bolærne	0.674	8.129	0.007	0.768	0.791
Flødevigen	0.673	8.146	0.011	0.790	0.791
Mandal	0.661	8.180	0.037	0.004	0.011

Investigating the spatial patterns of genetic structure in detail, the highest barriers to gene flow was found between: Bolærne, Flødevigen; Bolærne, Tisler; and Tisler, Flødevigen. These discontinuities were identified as the largest by 6, 5 and 5 loci. Concordantly, the overall pairwise F_{ST} matrix also supported the Bolærne, Tisler; and Bolærne, Flødevigen barrier as the largest (Fig.

6B). Pairwise F_{ST} across: Bolærne, Tisler; and Bolærne, Flødevigen barriers were estimated to be 0.002 and 0.001. Allele frequencies also varied significantly between these pairs (with p-values of 0.04 and 0.01 respectively). However, after FDR corrections, no pairwise comparisons of allele frequencies were found statistically different.

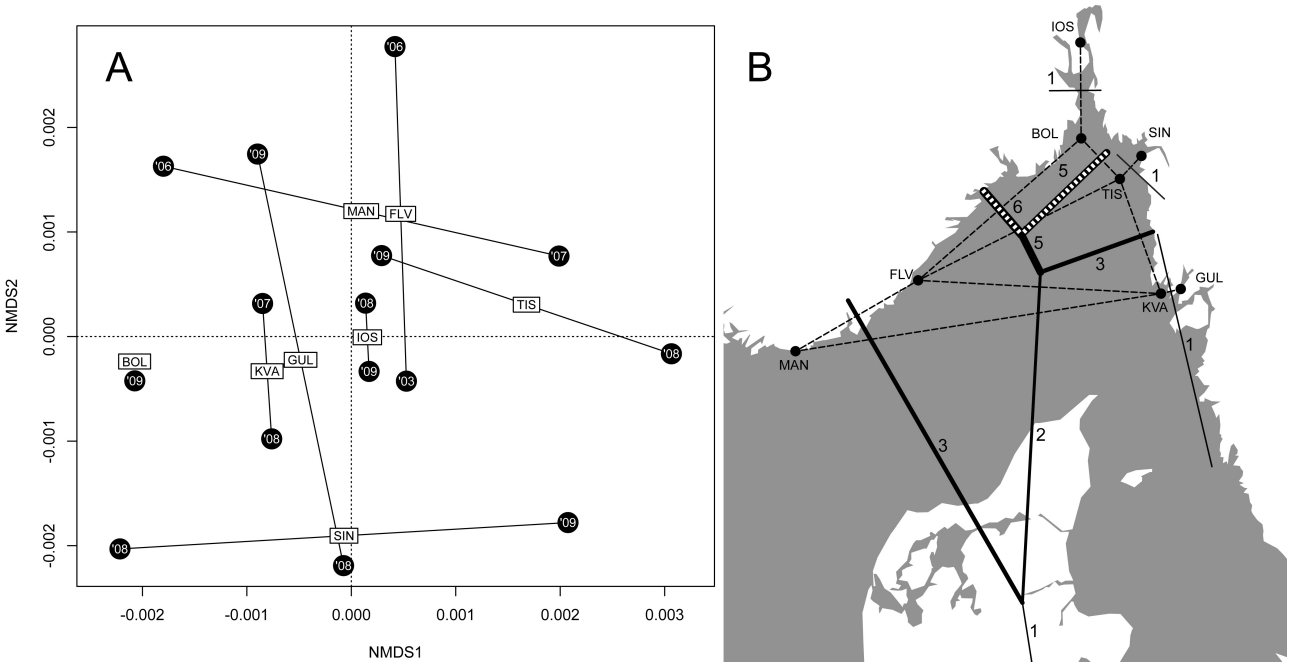

Figure 6 (A) NMDS diagram of pairwise F_{ST} matrix, where lines connect temporally replicated samples taken in Mandal (MAN), Flødevigen (FLV), Kåvra (KVA), Gullmaren (GUL), Tisler (TIS), Singlefjorden (SIN), Bolærne (BOL) and inner Oslofjorden (IOS). Numbers within black circles indicates when samplings were done. (B) Results from BARRIER analyses on 12 pairwise F_{ST} matrices, one for each locus. Black solid lines represents largest genetic barrier indicated by a locus, with a corresponding number of loci supporting the barrier. White broken line represents the largest barrier identified in the overall F_{ST} matrix. Black, broken lines represent the Delaunay triangulation between sites.

Discussion

This study used a combination of: acoustic telemetry, capture-mark-recapture studies, and microsatellite markers to elucidate: movement, spillover, and gene flow of lobsters within a network of experimental reserves. I report that only a small portion (3.6%) of lobsters tagged within reserves was traced beyond reserve boundaries, with a mean spillover distance of 3.7 km. In comparison, more than 36% were recaptured within reserves. Moreover, of the 30 lobsters monitored by acoustic telemetry in the Kvernskjær reserve, half of them were still there at the end of the study period (364 days). Thus probability of containing an individual within a reserve of 0.5 km^2 for one year was ≈ 50%. Taken together, lobsters in Skagerrak reserves displayed high site fidelity. Further, both telemetry and CMR studies suggested that male and female lobsters were equally contained within a reserve, as well as having equal spillover distances. However, male, and especially ovigerous female lobsters, used deeper habitats during the winter months. Male and ovigerous female lobsters also moved steeply to shallower habitats in spring, closely following the warming of upper water masses. In contrast, non-ovigerous female lobsters mostly used the same depth throughout the year. At first glance the sub-populations sampled for microsatellite markers appeared to have high connectivity, implied by the low overall F_{ST}. Number of effective migrants exchanged by sub-populations each generation was estimated to at least between 35 to 183 individuals, depending on effective population size. However, despite the high gene flow, allele frequency distributions varied significantly among geographic samples. A closer inspection of the spatial genetic patterns revealed a discontinuity of gene flow in the inner, northern part of Skagerrak. These findings are further discussed below.

Movement and spillover

Estimated spillover rate from three Skagerrak lobster reserves over six years was 3.6%. Compared to spiny lobster (*Palinurus elephas*) spillover from the marine reserves of Columbretes Islands (Goñi *et al.* 2010) and Su Pallosu (Follesa *et al*. 2011), located off Spanish Mediterranean coast and

western Sicily, Skagerrak spillover was very similar. Mean yearly spillover rate from the Columbretes reserve (44 km^2) was 3.7% and 6.7% for female and male spiny lobsters. Total spillover rate from Su Pallosu reserve (4 km^2) was 3.1% over 12 years. Furthermore, Su Pallosu spillover persisted beyond 20 km of reserve boundary, though 73% of recaptures were done within the first 5 km. In comparison, 73% of Skagerrak spillover lobsters were recaptured within 3.9 km of reserve boundaries, whereas all Columbretes spillover lobsters were caught within 4 km. The relatively truncated spillover distribution from the Columbretes reserve was credited to a discontinuity in favoured habitat of the spiny lobster (Goñi *et al.* 2006). Furthermore, outside the Lundy marine reserve (4 km^2) in the UK, there was a 140% increase in abundance of sub-legal sized European lobsters after four years of protection (Hoskin *et al.* 2011). This increase in sub-legal sized lobsters outside reserve was credited to spillover. Of all lobsters T-bar tagged within Skagerrak reserves, almost 46% were below legal catch size. Since Skagerrak fishers did not 'sample' for sub-legal sized lobsters, the spillover rate reported in this study could be a gross underestimation. Added to that: underreporting by fishers, tag loss, and the capture probability being less than 100% could substantially deflate estimated spillover rate. Moreover, the capture location reported by fishers could be uncertain, and their 'sampling' effort could have been biased towards fishing close to reserve boundaries, making data unrepresentative. However, the seemingly moderate spillover rate is concordant with a high recapture rate (36%) within reserves. Additionally, 50% of telemetry lobsters were contained within the Kvernskjær reserve for a year. I regard this as a conservative estimate at most (as discussed in next paragraph). The European lobster has also shown high site fidelity within the Flødevigen reserve. Over an approximately 200-day period, 95% utilization distributions of lobsters were between 5728 m^2 to 41548 m^2 (n = 18, mean 19876 m^2 ± 2152 SE) (Moland *et al.* 2011a). Net distance moved by these 18 lobsters, from release point to last tracking position, was only 8 m to 379 m.

Most losses of ovigerous females from telemetry study happened in late summer, subsequent to the hatching season. This steep loss of ovigerous females could be because of

moulting, as moulting in ovigerous females usually follows hatching of their eggs (Agnalt *et al.* 2007). On the other hand, if female moulting generally follows hatching, moulting as a possible causation for loss in other seasons can be dismissed. As a further matter, if lobsters in general were mostly lost from the telemetry study by moulting, we should have lost the later staged individuals (e.g. D_1 and D_2) at the very start of the study, followed by the subsequent moult stages. In American lobsters (*H. americanus*), nevertheless, a plateau in the progression of moulting is common during D_0. After the onset of D_1 there are no more plateaus, and it takes approximately 34-42, 24-20 and 16-22 (95% confidence limits) days until ecdysis. These predictions are applicable at temperatures 10°C, 15°C and 19°C respectively (Aiken 1973). However, this pattern was not explicitly observed. A large part of this unexplained variation could be a lack of experience in moult staging them before start of the study. Another part of the variation could have been created when each individual's "fate" was categorized based on depth data. However, I consider the assumptions made when deciding their fate to be quite conservative. Also, the sample size was probably too small to predict loss curves for each moult stage at study start. To conclude, I cannot be certain what caused male losses, or female losses outside hatching season. Movement out of the reserve could be a possibility. Moreover, censoring rates were high in spring, summer and fall, with few censoring events in winter. Concordantly, activity patterns of lobsters are generally high throughout the time period spring to fall, and relatively low in winter (Moland *et al.* 2011b). A simple explanation for high censoring rates could hence be physical damage to transmitters during periods of high activity. For example when they move in and out of their dens or in territorial disputes. The transmitters used were designed for surgical implementation in the abdominal cavity of fish, and not for exterior attachment. Hence, in future studies using exteriorly attached transmitters, efforts should be made to prevent damage to the transmitters. Although, a trade off between protective measures taken for the transmitters versus impediment to the lobsters is to be expected.

Predictions from the GAMM suggested a seasonal movement of male and particularly ovigerous female lobsters to deeper habitats in winter and shallower habitats in summer. American

lobsters on Georges Bank have also shown this seasonal movement. In comparison, American lobsters used mean depths of 100 m in summer, down to a mean depth of 400 m in winter (Cooper and Uzmann 1971). Distance moved during their seasonal migration was between 28 km and 77 km, depending on location. Growth increments were larger, and moult frequencies were higher for migrating lobsters, in contrast to stationary lobsters. The rationale for this behaviour was to maximise time spent in warmer water, increasing growth. Furthermore, ovigerous American lobsters tagged around Grand Manan (an island in Gulf of Maine/Bay of Fundy) moved down into Grand Manan basin (just east of Grand Manan) in winter. In spring, they were again caught in the shallows, in close proximity to release site. Movement rate peaked at 0.6 km d^{-1} in December and 0.5 km d^{-1} in June to July (Campbell 1986). Allegedly, this seasonal movement yields a more stable temperature, ensuring continuous development of embryos (Cowan *et al.* 2007). In addition, stationary ovigerous American lobsters would not experience sufficient degree-days to complete embryonic development (Campbell & Stasko 1986). Conversely, Kvernskjær lobsters did not have to move very far to attain deeper, warmer habitat in winter. Predicted 3.4°C isoline (where embryonic development halts, Campbell & Stasko 1986) did not reach beyond ≈ 14 m in telemetry study area. I propose that, in contrast to American lobsters migrating tens of kilometres every year, Kvernskjær lobsters only have to move small distances to experience preferred temperatures. An alternative explanation for the difference in seasonal depth use among groups could be intra-specific competition, as larger lobsters have a tendency to avoid areas of high lobster density (Steneck 2006). However, in the preliminary model fitting, lobster size did not explain a significant part of the variation in the data. Though, considering our relatively limited sample size, we cannot preclude size having an effect.

Gene flow in Skagerrak

Genetic structure among geographic samples was weak (F_{ST} = 0.000) with high variation in F_{ST} both within, and among loci. Yet, the allele frequencies varied significantly among geographic samples (p = 0.039). Deciphering this weak, variable signal of differentiation demands attentiveness (Waples 1998). First, the inherent bias added by non-random sampling has to be disentangled from any underlying genetic signal of differentiation. Second, if the observed pattern is consistent among temporally replicated samples, it probably reflects an underlying signal of differentiation. An expected bias as result of sampling error can be described in a statistical sense, and decreases with sample size S, approximately by 1/(2S) (Wright 1978). I consider a sample size of 718 individuals to suffice, yielding an expected bias of 0.0007. Further, our samplings were temporally replicated, ensuring that several cohorts were sampled. Although no significant differences in allele frequencies were found between temporal samples, and the AMOVA did not partition a significant amount of variation between them, the NMDS diagram did not convey a consistent pattern over time. Another potential source of bias affecting the estimate of F_{ST} is natural selection working on, or nearby, sampled loci. Directional selection upwardly biases F_{ST}, whereas balancing selection downwardly bias F_{ST} (Beaumont 2008; Nielsen 2005b). For example, in three-spined sticklebacks (*Gasterosteus aculeatus*), up to 15% of 'uniformly' sampled loci have shown signs of balancing selection, while nearly 3% have been candidates for directional selection (Mäkinen *et al*. 2008). Although natural selection was excluded during preliminary analyses, weak associations with loci under selection could explain some inter-locus variation in estimated F_{ST}. Moreover, compared to other high gene flow species in Skagerrak, overall estimated F_{ST} was low. Both cod (Knutsen *et al.* 2003; Jorde *et al.* 2007) and brown crab (*Cancer pagurus*) (Ungfors *et al.* 2009) sampled within Skagerrak have higher estimated F_{ST}. However, a recent study on cod in Skagerrak has shown that such small F_{ST} values probably are of biological relevance (Knutsen et al. 2011). Hence, the F_{ST} reported in this study could be a true biological signal. Besides, sampling of lobster mitochondrial DNA has revealed significant genetic differences already between samples taken inside Skagerrak

(e.g. Swedish Skagerrak coast, Drøbak, and Mandal) and immediately outside (e.g. western Norway, England, and Germany) (Triantafyllidis *et al.* 2005).

Gene flow appeared to be most restricted between sample sites in northern Skagerrak. From a biophysical modelling study of American lobsters in Gulf of Main, Incze *et al.* (2010) reported self-recruitment rate to vary between a few percentages to over 90% within domains. Most of this variation was accredited to a domain's position relative to the prevailing currents. Thus a possible explanation for restricted gene flow in northern Skagerrak could be a less prominent 'Skagerrak gyre' in northern Skagerrak, resulting in stronger retention of larvae. However, this statement is highly speculative, and further studies are needed to elucidate on retention patterns of lobster larvae in Skagerrak. For example, conducting a full blown biophysical modelling study (e.g. Incze *et al.* 2010) would greatly add to our knowledge on this. Such a study could also potentially help to pinpoint where reserves should be placed to maximise: dispersal of lobster larvae into fished areas, and exchange of larvae among reserves. An alternative explanation for this barrier to gene flow in northern Skagerrak could be limited post-settlement dispersal across the outer Oslofjord, as water depths of more than 200 m separate each side of the fjord. European lobsters are not known to traverse such depths. For example, no T-bar tagged lobsters from the Bolærne reserve was recaptured on the eastern side of the Oslofjord, despite the seemingly short distance across (< 8 km). In comparison, 10% of Bolærne spillover lobsters were recaptured beyond 8 km on the western side. Conversely, disentangling gene flow occurring at two distinct life history stages of the lobster is like shuffling a sorted deck of cards. First lightly, then heartily, and subsequently deciding which shuffling event made the pattern you were dealt. Likewise, gene flow through adult dispersal, but mostly though drift of larvae with the NCW current, permute any underlying genetic structure.

Synthesising movement, spillover, and gene flow: telemetry and T-bar tagged lobsters showed very high site fidelity, with odd movement distances up to 25 km. Furthermore, mean post-settlement dispersal of European lobsters (until legal catch size, 85 mm CL) has been estimated to less than 6 km (Bannister *et al*. 1994). Combined with present estimates, this gives a good

approximation of benthic phase dispersal of lobsters. Movement occurring over longer distances (>25 km) can hence be accredited to larval dispersal. Palumbi (2003) argue that mean dispersal distances of pelagic marine larvae can be estimated through the slope of an isolation by distance model. According to his algorithm, pairwise F_{ST} increments of 0.01 to 0.07 per 1000 km would yield mean larval dispersal distances of 150 km to 25 km. These estimates are based on diverse taxa, from common sole (*Solea vulgaris*) to tropical periwinkle (*Littorina cingulata*). Although Skagerrak lobsters' pairwise F_{ST} did not show an increasing trend, mean dispersal distance of lobster larvae most probably lie somewhere between these estimates (25km - 150km). Adopting Palumbi's (2004) terminology: the lobster's 'spillover cloud' only reaches within our sampling domains (<25km), whereas their 'larval neighbourhood' most probably extend across domains (25km - 150km). Complementarily, assuming an effective population size of 500 in each sampled sub-population, and given a F_{ST} of at most 0.001 (estimated lower 95% CI), the Island model would predict an exchange of at least 146 effective migrants per generation. Equivalently, given these assumptions, migrants would constitute at least ≈ 29% of sub-population's effective population size. By analogy, if a reserve had been placed within each sub-population, holding a significant portion of the sub-population's effective population size, the reserves would ultimately exchange migrants. Increasing effective population sizes by increasing reserve sizes would only increase the probability of exchange. It should be noted that many assumptions of the Island model were violated here, and no good estimates of true lobster effective population sizes within Skagerrak exists. Added to that, there was a high inter-locus variation in estimated F_{ST}, yielding inaccurate estimates of gene flow. Hence, my estimates of N_em have to be treated with great caution. However, because this extrapolation is of such importance to managers (Palumbi 2003), these estimates may at least be used as hints for the demographic effects of the observed level of gene flow (Waples 1998).

Management implications and future directions

Taken together, the lobster's high site fidelity suggests that future reserves could be of almost any size (≥ 0.5 km^2), and still contain a substantial number of lobsters over extended time periods. Compared to other lobster species within reserves, European lobster spillover was similar in both magnitude and extent. Moreover, lobster reserves should be placed strategically regarding deeper habitats, used by males and ovigerous females during winter. This could potentially enhance movement out of reserves, increasing spillover. Further, due to the high connectivity of lobsters in Skagerrak, reserves could be relatively distantly spaced (≥ 300 km) and still (hypothetically) exchange a demographically relevant number of migrants, given large effective population sizes within large reserves. However, a decreasing gradient in connectivity is to be expected as you move away from major currents. This study was bound by the limitations of using gene flow as an approximation for small-scale dispersal patterns over short time frames. Thus further studies are needed on lobster larvae retention patterns to pinpoint where reserves should be placed, to maximise recruitment benefits and connectivity among reserves.

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